CLEAN PAGES OF SPECIFICATION

EXAMPLE 1

Selection of TGC-480 from the data base and analysis of the base sequence

Among the EST database supplied by Smithkline Beecham LTD. (SB), the clone supposed to code the signal sequence contributing to secretion and the processing region were selected. In more detail, the DNA sequences of the EST were translated to the corresponding amino acid sequences and then the clone having the cluster of the hydrophobic amino acids (Leu, Ile, Val, Ala, etc.) after Met and further carrying the reserved sequence (Arg-Arg, Lys-Ara, Lys-Lys) at the processing region within the same frame was selected. As the result, HGS:558273 was discovered as the EST clone that satisfied these conditions. However, taking into account the fact that there was a possibility for the EST sequence to have deletion, insertion and miscoding of the base sequence as well as the fact that the EST sequence is a partial sequence of cDNA, SB was kindly requested to send this clone as TGC-480 to us and the whole base sequence of the insertion DNA fragment in the plasmid was determined by using fluorescent DNA sequencer (ABI PRINSMTH 377, Perkin Elmer). The results indicated that the insertion sequence (cDNA) of the clone carried the open reading frame for 378 bases as represented by SEQ ID NO: 16, which encoded the polypeptide comprising 125 amino acid residue as shown by SEQ ID NO:1. The residue consisting of 21 amino acids at N-terminus as shown by SEQ ID NO: 1 was anticipated to be the signal sequence for secretion. It was also verified that this clone expressed in brain, testis, heart, etc.

The cDNA fragment could be obtained by conventional methods, based on the above information. In other words, using the base sequence as described in SEQ ID NO:16, the primers for PCR corresponding to 5' end and 3' end (e.g.,

ATGGCCAAGTACCTGGCCCAGATCA (SEQ ID NO:31); and

TCACGTATGGGGCATCTGCCCTTTT) (SEQ ID NO:32); were prepared, followed by employment of the cDNA library of the above-stated tissues (e.g., brain, testis, heart, etc.) or RT-PCR with use of mRNA derived from the aforementioned tissues as a template to obtain the cDNA fragment. In detail, the solution 50 μl containing 5 pmol of the each primer, 5 μl of 100 mM Tris-HC1 buffer (pH9.0), 5 μl of 500 mM potassium chloride solution, 3 μl of 25 mM magnesium chloride solution, 4 μl of 2.5 mM deoxyribonucleotide solution, 1 μl of cDNA solution, and 0.5 μl of TaKaRa Tag™ was prepared. The PCR reaction was performed according to the following program, namely, the resultant solution was placed at 95°C for 1 minute in TaKaRa PCR Thermal Cycler MP (Takara shuzo Co.,Ltd.), then at 95°C for 30 seconds, at 65°C for 1 minute and at 72°C for 2 minutes; this cycle was repeated 35 times in total and further reacted at 72°C for 10 minutes to obtain the target PCR fragment.

EXAMPLE 2

Selection of TGC-546 from the data base and analysis of the base sequence

After selection of HGS:447917 by the similar method as described in EXAMPLE l, this clone was received as TGC-546 and then the base sequence was confirmed. As the result, it was revealed that the insertion sequence (cDNA) of the clone carried the open reading frame for 366 bases as represented by SEQ ID NO: 17, which encoded the polypeptide comprising 121 amino acid residue as represented by SEQ ID NO:2. The residue consisting of 23 amino acids at N-terminus of the amino acid sequence as represented by SEQ ID NO:2 was anticipated to be the signal sequence for secretion. It was also verified that this clone expressed in epididymis.

The cDNA fragment could be obtained by conventional methods, based on the above information. In other words, using the base sequence as described in SEQ ID NO: 17, the primers for PCR corresponding to 5' end and 3' end (e.g., ATGCACAGATCAGAGCCATTTCTGA (SEQ ID NO:33); and TTACAGTAGTGGCAGTAACACTTGG) (SEQ ID NO:34); were prepared, followed by employment of the cDNA library of the above-stated tissues (e.g., dpididymis, etc.) or RT-PCR with use of mRNA derived from the aforementioned tissues as a template to obtain the cDNA fragment. The reaction conditions to be applied to PCR were those as described in EXAMPLE 1.

EXAMPLE 3

Selection of TGC-595 from the data base and analysis of the base sequence

After selection of HGS:1006634 by the similar method as described in EXAMPLE 1, this clone was received as TGC-595 and then the base sequence was confirmed. As the result, it was revealed that the insertion sequence (cDNA) of the clone carried the open reading frame for 672 bases as referred to as SEQ ID NO:18, which encoded the polypeptide comprising 223 amino acid residue as shown by SEQ ID NO:3. The residue consisting of 19 amino acids at N-terminus of the amino acid sequence as shown by SEQ ID NO:3 was anticipated to be the signal sequence for secretion. It was also verified that this clone expressed in spinal cord, T cells, retina, etc.

The cDNA fragment could be obtained by conventional methods, based on the above information. In other words, using the base sequence as described in SEQ ID NO:18, the primers for PCR corresponding to 5' end and 3' end (e.g., ATGAAGTTCGTCCCCTGCCTCCTGC (SEQ ID NO:35); and

TCACCCTCGGAAGAAGCTGATGAGA) (SEQ ID NO:36); were prepared, followed by employment of the cDNA library of the above-stated tissues (e.g., spinal cord, T cells, retina, etc.) or RT-PCR with use of mRNA derived from the aforementioned tissues as a template to obtain the cDNA fragment. The reaction conditions to be applied to PCR were those as described in EXAMPLE 1.

EXAMPLE 4

Selection of TGC-623 from the data base and analysis of the base sequence

After selection of HGS:92551 by the similar method as described in EXAMPLE 1, this clone was received as TGC-623 and then the base sequence was confirmed. As the result, it was revealed that the insertion sequence (cDNA) of the clone carried the open reading frame for 747 bases as referred to as SEQ ID NO: 19, which encoded the polypeptide comprising 248 amino acid residue as shown by SEQ ID NO:4. The residue consisting of 21 amino acids at N-terminus of the amino acid sequence as shown by SEQ ID NO:4 was anticipated to be the signal sequence for secretion. It was also verified that this clone expressed in cerebellum, adrenal, etc.

The cDNA fragment could be obtained by conventional methods, based on the above information. In other words, using the base sequence as described in SEQ ID NO:19, the primers for PCR corresponding to 5' end and 3' end (e.g., ATGGGACCTGTGCGGTTGGGAATAT (SEQ ID NO:37); and TCAAAGATCTTCTCGGTCAAGTTTG) (SEQ ID NO:38); were prepared, followed by employment of the cDNA library of the above-stated tissues (e.g., cerebellum, adrenal, etc.) or RT-PCR with use of mRNA derived from the aforementioned tissues

as a template to obtain the cDNA fragment. The reaction conditions to be applied to PCR were those as described in EXAMPLE 1.

EXAMPLE 5

Selection of TGC-624 from the data base and analysis of the base sequence

After selection of HGS:1731120 by the similar method as described in EXAMPLE 1, this clone was received as TGC-624 and then the base sequence was confirmed. As the result, it was revealed that the insertion sequence (cDNA) of the clone carried the open reading frame for 522 bases as referred to as SEQ ID NO:20, which encoded the polypeptide comprising 173 amino acid residue as shown by SEQ ID NO:5. The residue consisting of 19 amino acids at N-terminus of the amino acid sequence as shown by SEQ ID NO:5 was anticipated to be the signal sequence for secretion. It was also verified that this clone expressed in dendritic cells, T cells, etc.

The cDNA fragment could be obtained by conventional methods, based on the above information. In other words, using the base sequence as described in SEQ ID NO:20, the primers for PCR corresponding to 5' end and 3' end (e.g., ATGTTTTGCCCACTGAAACTCATCC (SEQ ID NO:39); and TCATGAAAATATCCATTCTACCTTG) (SEQ ID NO:40); were prepared, followed by employment of the cDNA library of the above-stated tissues (e.g., dendritic cells, T cells, etc.) or RT-PCR with use of mRNA derived from the aforementioned tissues as a template to obtain the cDNA fragment. The reaction conditions to be applied to PCR were those as described in EXAMPLE 1.

EXAMPLE 6

Selection of TGC-625 from the data base and analysis of the base sequence

After selection of HGS:1014817 by the similar method as described in EXAMPLE 1, this clone was received as TGC-625 and then the base sequence was confirmed. As the result, it was revealed that the insertion sequence (cDNA) of the clone carried the open reading frame for 786 bases as referred to as SEQ ID NO:21, which encoded the polypeptide comprising 261 amino acid residue as shown by SEQ ID NO:6. The residue consisting of 20 amino acids at N-terminus of the amino acid sequence as shown by SEQ ID NO:6 was anticipated to be the signal sequence for secretion. It was also verified that this clone expressed in vascular endothelial cells, bone marrow, etc.

The cDNA fragment could be obtained by conventional methods, based on the above information. In other words, using the base sequence as described in SEQ ID NO:21, the primers for PCR corresponding to 5' end and 3' end (e.g., ATGGAACTGCTTCAAGTGACCATTC (SEQ ID NO:41); and TCAGTTCTTGGTTTTTCCTTGTGCA) (SEQ ID NO:42); were prepared, followed by employment of the cDNA library of the above-stated tissues (e.g., vascular endothelial cells, bone marrow, etc.) or RT-PCR with use of mRNA derived from the aforementioned tissues as a template to obtain the cDNA fragment. The reaction conditions to be applied to PCR were those as described in EXAMPLE 1.

EXAMPLE 7

Selection of TGC-628 from the data base and analysis of the base sequence

After selection of HGS:1878022 by the similar method as described in EXAMPLE 1, this clone was received as TGC-628 and then the base sequence was confirmed. As the result, it was revealed that the insertion sequence (cDNA) of the clone carried the open reading frame for 732 bases as referred to as SEQ ID) NO:22, which encoded the polypeptide comprising 243

amino acid residue as shown by SEQ ID NO:7. The residue consisting of 30 amino acids at N-terminus of the amino acid sequence as shown by SEQ ID NO:7 was anticipated to be the signal sequence for secretion. It was also verified that this clone expressed in thymus, placenta, etc.

The cDNA fragment could be obtained by conventional methods, based on the above information. In other words, using the base sequence as described in SEQ ID NO:22, the primers for PCR corresponding to 5' end and 3' end (e.g., ATGCGACCCCAGGGCCCCGCCGCCT (SEQ ID NO:43); and TTATTTTGGTAGTTCTTCAATAATG) (SEQ ID NO:44); were prepared, followed by employment of the cDNA library of the above-stated tissues (e.g., thymus, placenta, etc.) or RT-PCR with use of mRNA derived from the aforementioned tissues as a template to obtain the cDNA fragment. The reaction conditions to be applied to PCR were those as described in EXAMPLE 1.

EXAMPLE 8

Selection of TGC-708 from the data base and analysis of the base sequence

After selection of HGS:2346555 by the similar method as described in EXAMPLE 1, this clone was received as TGC-708 and then the base sequence was confirmed. As the result, it was revealed that the insertion sequence (cDNA) of the clone carried the open reading frame for 450 bases as referred to as SEQ ID NO:23, which encoded the polypeptide comprising 149 amino acid residue as shown by SEQ ID NO:8. The residue consisting of 18 amino acids at N-terminus of the amino acid sequence as shown by SEQ ID NO:8 was anticipated to be the signal sequence for secretion. It was also verified that this clone expressed in monocytes.

The cDNA fragment could be obtained by conventional methods, based on the above information. In other words, using the base sequence as described in SEQ ID NO:23, the primers for PCR corresponding to 5' end and 3' end (e.g., ATGAAGTTACAGTGTGTTfCCCTTT (SEQ ID NO:45); and TCAGGAGGCCGATGGGGGCCAGCAC) (SEQ ID NO:46); were prepared, followed by employment of the cDNA library of the above-stated tissues (e.g., monocytes, etc.) or RT-PCR with use of mRNA derived from the aforementioned tissues as a template to obtain the cDNA fragment. The reaction conditions to be applied to PCR were those as described in EXAMPLE 1.

EXAMPLE 9

Selection of TGC-711 from the data base and analysis of the base sequence

After selection of HGS:809616 by the similar method as described in EXAMPLE 1, this clone was received as TGC-711 and then the base sequence was confirmed. As the result, it was revealed that the insertion sequence (cDNA) of the clone carried the open reading frame for 411 bases as referred to as SEQ ID NO:24, which encoded the polypeptide comprising 136 amino acid residue as shown by SEQ ID NO:9. The residue consisting of 20 amino acids at N-terminus of the amino acid sequence as shown by SEQ ID NO:9 was anticipated to be the signal sequence for secretion. It was also verified that this clone expressed in cerebellum, lung, etc.

The cDNA fragment could be obtained by conventional methods, based on the above information. In other- words, using the base sequence as described in SEQ ID NO:24, the primers for PCR corresponding to 5' end and 3' end (e.g., ATGGCCAGCCTGGGGCTGCTGCTCC (SEQ ID NO:47); and

TCATGAGGCTCCTGCAGAGGTCTGA) (SEQ ID NO:48); were prepared, followed by employment of the cDNA library of the above-stated tissues (e.g., cerebellum, lung, etc.) or RT-PCR with use of mRNA derived from the aforementioned tissues as a template to obtain the cDNA fragment. The reaction conditions to be applied to PCR were those as described in EXAMPLE 1.

EXAMPLE 10

Selection of TGC-714 from the data base and analysis of the base sequence

After selection of HGS:1260352 by the similar method as described in EXAMPLE 1, this clone was received as TGC-714 and then the base sequence was confirmed. As the result, it was revealed that the insertion sequence (cDNA) of the clone carried the open reading frame for 372 bases as referred to as SEQ ID NO:25, which encoded the polypeptide comprising 123 amino acid residue as shown by SEQ ID NO:10. The residue consisting of 22 amino acids at N-terminus of the amino acid sequence as shown by SEQ ID NO: 10 was anticipated to be the signal sequence for secretion. It was also verified that this clone expressed in epididymis.

The cDNA fragment could be obtained by conventional methods, based on the above information. In other words, using the base sequence as described in SEQ ID NO:25, the primers for PCR corresponding to 5' end and 3' end (e.g., ATGAAACTCCTGCTGCTGGCTCTTC (SEQ ID NO:49); and TCATGAGCTATGGTGAACATTTGGA) (SEQ ID NO:50); were prepared, followed by employment of the cDNA library of the above-stated tissues (e.g., epididymis, etc.) or RT-PCR with use of mRNA derived from the aforementioned tissues as a template to obtain the cDNA fragment. The reaction conditions to be applied to PCR were those as described in EXAMPLE 1.

EXAMPLE 11

Selection of TGC-715 from the data base and analysis of the base sequence

After selection of HGS:81772 by the similar method as described in EXAMPLE 1, this clone was received as TGC-715 and then the base sequence was confirmed. As the result, it was revealed that the insertion sequence (cDNA) of the clone carried the open reading frame for 492 bases as referred to as SEQ ID NO:26, which encoded the polypeptide comprising 163 amino acid residue as shown by SEQ ID NO:11. The residue consisting of 20 amino acids at N-terminus of the amino acid sequence as shown by SEQ ID NO: 11 was anticipated to be the signal sequence for secretion. It was also verified that this clone expressed in epididymis.

The cDNA fragment could be obtained by conventional methods, based on the above information. In other words, using the base sequence as described in SEQ ID NO:26, the primers for PCR corresponding to 5' end and 3' end (e.g., ATGGGCGGCCTGCTGCTGGCTGCTT (SEQ ID NO:51); and CTACTGTGACAGGAAGCCCAGGCTC) (SEQ ID NO:52); were prepared, followed by employment of the cDNA library of the above-stated tissues (e.g., epididymis, etc.) or RT-PCR with use of mRNA derived from the aforementioned tissues as a template to obtain the cDNA fragment. The reaction conditions to be applied to PCR were those as described in EXAMPLE 1.

EXAMPLE 12

Selection of TGC-749 from the data base and analysis of the base sequence

After selection of HGS:1379897 by the similar method as described in EXAMPLE 1, this clone was received as TGC-749 and then the base sequence was confirmed. As the

result, it was revealed that the insertion sequence (cDNA) of the clone carried the open reading frame for 906 bases as referred to as SEQ ID) NO:27, which encoded the polypeptide comprising 301 amino acid residue as shown by SEQ ID NO: 12. The residue consisting of 18 amino acids at N-terminus of the amino acid sequence as shown by SEQ ID NO: 12 was anticipated to be the signal sequence for secretion. It was also verified that this clone expressed in T cells, placenta, liver, large intestine, etc.

The cDNA fragment could be obtained by conventional methods, based on the above information. In other words, using the base sequence as described in SEQ ID NO:27, the primers for PCR corresponding to 5' end and 3' end (e.g., ATGGCCCGGCATGGGTTACCGCTGC (SEQ ID NO:53); and TTACAGCTCCCCTGGCGGCCGGCCT) (SEQ ID NO:54); were prepared, followed by employment of the cDNA library of the above-stated tissues (e.g., T cells, placenta, liver, large intestine, etc.) or RT-PCR with use of mRNA derived from the aforementioned tissues as a template to obtain the cDNA fragment. The reaction conditions to be applied to PCR were those as described in EXAMPLE 1.

EXAMPLE 13

Selection of TGC-768 from the data base and analysis of the base sequence

After selection of HGS:398232 by the similar method as described in EXAMPLE 1, this clone was received as TGC-768 and then the base sequence was confirmed. As the result, it was revealed that the insertion sequence (cDNA) of the clone carved the open reading frame for 210 bases as referred to as SEQ ID N0:28, which encoded the polypeptide comprising 69 amino acid residue as shown by SEQ ID NO:13. The residue consisting of 26 amino acids at

N-terminus of the amino acid sequence as shown by SEQ ID NO:13 was anticipated to be the signal sequence for secretion. It was also verified that this clone expressed in testis.

The cDNA fragment could be obtained by conventional methods, based on the above information. In other words, using the base sequence as described in SEQ ID NO:28, the primers for PCR corresponding to 5' end and 3' end (e.g., ATGTGCTGGCTGCGGGCATGGGGCC (SEQ ID NO:55); and TTATCTATTCATCATATATTTCTTA) (SEQ ID NO:56); were prepared, followed by employment of the cDNA library of the above-stated tissues (e.g., testis, etc.) or RT-PCR with use of mRNA derived from the aforementioned tissues as a template to obtain the cDNA fragment. The reaction conditions to be applied to PCR were those as described in EXAMPLE 1.

EXAMPLE 14

Selection of TGC-772 from the data base and analysis of the base sequence

After selection of HGS:2079036 by the similar method as described in EXAMPLE 1, this clone was received as TGC-772 and then the base sequence was confirmed. As the result, it was revealed that the insertion sequence (cDNA) of the clone carried the open reading frame for 210 bases as referred to as SEQ ID NO:29, which encoded the polypeptide comprising 69 amino acid residue as shown by SEQ ID NO:14. The residue consisting of 23 amino acids at N-terminus of the amino acid sequence as shown by SEQ ID NO:14 was anticipated to be. the signal sequence for secretion. It was also verified that this clone expressed in pancreas, placenta, etc.

The cDNA fragment could be obtained by conventional methods, based on the above information. In other words, using the base sequence as described in SEQ ID NO:29, the

primers for PCR corresponding to 5' end and 3' end (e.g., ATGGGGTTCCCGGCCGCGGCGCTGC (SEQ ID NO:57); and CTACGCCGAGACCGTGGGCCTGCGG) (SEQ ID NO:58); were prepared, followed by employment of the cDNA library of the above-stated tissues (e.g., pancreas, placenta, etc.) or RT-PCR with use of mRNA derived from the aforementioned tissues as a template to obtain the cDNA fragment. The reaction conditions to be applied to PCR were those as described in EXAMPLE 1.

EXAMPLE 15

Selection of TGC-790 from the data base and analysis of the base sequence

After selection of HGS:2450362 by the similar method as described in EXAMPLE 1, this clone was received as TGC-790 and then the base sequence was confirmed. As the result, it was revealed that the insertion sequence (cDNA) of the clone carried the open reading frame for 594 bases as referred to as SEQ ID NO:30, which encoded the polypeptide comprising 197 amino acid residue as shown by SEQ ID NO:15. The residue consisting of 26 amino acids at N-terminus of the amino acid sequence as shown by SEQ ID NO: 15 was anticipated to be the signal sequence for secretion. It was also verified that this clone expressed in placenta, etc. The cDNA fragment could be obtained by conventional methods, based on the above information. In other words, using the base sequence as described in SEQ ID N0:30, the primers for PCR corresponding to 5' end and 3' end (e.g., ATGCGAGGTGGCAAATGCAACATGC (SEQ ID NO:59); and TCATAAACTTGTGTTGGGCTTTAGG) (SEQ ID NO:60); were prepared, followed by employment of the cDNA library of the above-stated tissues (e.g., placenta, etc.) or RT-PCR with use of mRNA derived from the aforementioned tissues as a template to obtain the cDNA

fragment. The reaction conditions to be applied to PCR were those as described in EXAMPLE 1.

EXAMPLE 16

Secretion expression of TGC-480 product in COS7 cells

The expression vector to express TGC-480 product in animal cells was obtained by insertion of the DNA fragment containing ORF encoding the TGC-480 product into the expression vector pCAN618 for animal cells.

First of all, the PCR was performed by using the synthetic DNA [5'-ACGCTCGAGTTACTTGTCATCGTCGTCCTTGTAGTCCGTATGGGGCATCT GCCCTTTTTC-3' : (SEQ ID NO:62)], which was designed to locate the synthetic DNA [5'-TCGGAATTCGCCATGGCCAAGTACCTGGCCCAGATC-3' : (SEQ ID NO:61)] having the recognition site for the restriction enzyme Eco RI immediately before the translation start codon initiator, the FLAG sequence consisting of 8 amino acids (Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys) (SEQ ID NO:71); at C-terminus of the TGC-480 protein, and subsequent stop codon and the recognition site for the restriction enzyme Xho I, by using the cDNA fragment encoding the TGC-480 protein obtained in EXAMPLE 1 as a template. The PCR reaction was performed according the following program, with use of Pyrobest DNA Polymerase (Takara Shuzo Co.,. Ltd.); namely, the reaction mixture was placed at 94°C for 1 minute, then at 98°C for 10 seconds, at 60°C for 30 seconds and at 72°C for 1 minute; this cycle was repeated 25 times in total. Finally, extension reaction at 72°C for 10 minutes was performed to obtain the DNA fragment containing the ORF of TGC-480. The resultant DNA fragment was cleaved with the restriction enzyme Eco RI and Xho 1, followed by insertion of them into Eco

RI/Xho I sites in pCAN618 to obtain the expression vector for TGC-480 protein, pCAN618/TGC-480FLAG for animal cells.

In the DMEM (medium ; Gibco BRL) containing 10% FBS (bovine fetal serum) , $4x10^5$ COS7 cells were incubated in a 6-well plate for 24 hours. The expression vector pCAN618/TGC480FLAG DNA was introduced into these cells with use of LipofectAMINE (Gibco BRL) and then cultured for further 18 hours. The medium was replaced with Opti-MEM (Medium; Gibco BRL) containing 0.05 % CHAPS and cultured for further 24 hours, followed by recovery of the supernatant. The supernatant was subjected to centrifugation to remove the floating cells, and then condensed to 1/10 by ultrafiltration (Centricon; Amicon). The resultant solution was added with the same volume of SDS-Sample Buffer containing 2-mercaptoethanol, followed by electrophoresis in 10 - 25% SDS-PAGE (TEFCO). After transferring this to the PVDF membrane (Amersham), Western Blot analysis was performed. Anti-FLAG mouse IgG (10 μ g/ml ; Sigma) was used as the primary antibody while HRP (Horseraddish peroxidase)-labeled anti-mouse IgG antibody (x 2000 dilution; Amersham) was used as the secondary antibody, with use of ECLplus Western Blot Detection System (Amersham) for detection. As the result, it was revealed that TGC-480 protein was secreted into the culture supernatant (Fig. 1).

EXAMPLE 17

Secretion expression of TGC-623 product in COS7 cells

The expression vector to express TGC-623 product in animal cells was obtained by insertion of the DNA fragment containing ORF encoding the TGC-623 product into the expression vector pCAN618FLAG for animal cells. pCAN618FLAg was derived from the

plasmid vector pCAN618, and pCAN618FLAG can express the target protein as the FLAG fused protein, by coinciding the reading frame of the base sequence encoding the FLAG sequence for 8 amino acids existing immediately after Sal I site (Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys) (SEQ ID NO:71); to the end codon.

First of all, the PCR was performed by using the synthetic DNA [5'-AGGCAAGTCGACAAGATCTTCTCGGTCAAGTTTGGGGTGGCTTCCTGTC TTGGTCAT-3': (SEQ ID NO:68)], which was designed to locate the restriction enzyme Sal I recognition site at C end of TGC-623 protein, and the synthetic DNA [5'-TAGACGAATTCCCACCATGGGACCTGTGCGGTTGGGAATATTGC-3' (SEQ ID NO:67)] having the recognition site for the restriction enzyme Eco RI immediately before the translation start codon initiator by using the cDNA fragment encoding TGC-623 protein obtained in EXAMPLE 4. The PCR reaction was performed according to the following program, with use of Pyrobest DNA Polymerase (Takara); namely, the reaction mixture was placed at 94°C for 1 minute, then at 98°C for 10 seconds, at 57°C for 30 seconds and at 72° C for 1 minute; this cycle was repeated 25 times in total. Finally, extension reaction at 72°C for 10 minutes was performed to obtain the DNA fragment containing the ORF of TGC-623. The resultant DNA fragment was cleaved with the restriction enzyme Eco RI and Sal I, followed by insertion of them into Eco RI/Sal I sites in pCAN618FLAG to obtain the expression vector for TGC-623 protein, or pCAN618/TGC-623FLAG for animal cells.

This expression vector was introduced into COS7 cells in the similar manners for those in EXAMPLE 16, and the culture supernatant was prepared, thereby performing the Western

Blot analysis. As the result, it was revealed that TGC-623 protein was secreted into the culture supernatant.(Fig. 1)

EXAMPLE 18

Secretion expression of TGC-711 product in COS7 cells

The expression vector to express TGC-711 product in animal cells was obtained in the similar manner as those for TGC-480 product described in EXAMPLE 16.

First of all, the PCR was performed by using the synthetic DNA [5'-ACGCTCGAGTTACTTGTCATCGTCGTCCTTGTAGTCTGAGGCTCCTGCAG AGGTCTGAGA-3': (SEQ ID NO:64)], which was designed to locate the FLAG sequence (Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys) (SEQ ID NO:71); and subsequently the end codon as well as the recognition site for the restriction enzyme Xho I in this sequence order, and the synthetic DNA [5'-TCGGAATTCGCCATGGCCAGCCTGGGGCTGCTGCTC-3': (SEQ ID NO:63)] having as a template the recognition site for the restriction enzyme Eco RI immediately before the translation start codon initiator, by using the cDNA fragment encoding TGC-711 protein obtained in EXAMPLE 9 as a template. The PCR reaction and subsequent treatments were performed under the similar conditions as those in EXAMPLE 16, to obtain the expression vector for human TGC-711 protein, or pCAN618/TGC-711 FLAG for animal cells. This expression vector was introduced into COS7 cells in the similar manners for those in EXAMPLE 16, and the culture supernatant was prepared, thereby being used for performing the Western Blot analysis. As the result, it was revealed that TGC-711 protein was secreted into the culture supernatant (Fig. 1).

EXAMPLE 19

Secretion expression of TGC-714 product in COS7 cells

The expression vector to express TGC-714 product in animal cells was obtained in the similar manner as those for TGC-480 product described in EXAMPLE 16.

First of all, the PCR was performed by using the synthetic DNA [5'-ACGCTCGAGTTACTTGTCATCGTCGTCCTTGTAGTCTGAGCTATGGTGAA CATTTGGAAG-3': (SEQ ID NO:66)], which was designed to locate the FLAG sequence (Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys) (SEQ ID NO:71); and subsequently the end codon as well as the recognition site for the restriction enzyme Xho I in this sequence order, and